DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

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Research Proposal

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7.	**Title of Research Problem**	:	**EPIDEMIOLOGY AND BIORATIONAL MANAGEMENT OF TWISTER [(*Colletotrichum gloeosporioides*) (Penzig) Penzig and Saccardo] DISEASE OF ONION**

8. Introduction

The onion (*Allium cepa* L.) commonly known as the bulb onion or common onion. It belongs to the family *Amaryllidaceae* (previously classified under *Alliaceae*) and is a monocotyledonous, cross pollinated crop. The species has a diploid chromosome number of 2n = 16. Onion is a used in almost every kitchen because of its high valued pungency and nutrition and is considered as “Queen of the kitchen” (Selvaraj, 1976; Griffiths *et al*., 2002). The Latin term *cepa* translates to onion. In Persian language onion is called as *pyaz*. The primary centre of origin of onion is Central Asia while the near East and Mediterranean are the secondary centre of origin (Vavilov, 1951). It was introduced in India from Palestine (Yadav *et al.*, 2013). Onion is a vital spice and one of the most extensively cultivated vegetable crop across temperate, subtropical and tropical regions worldwide. The term "wild onion" is used for various *Allium* species, *A. cepa* is exclusively known from cultivation and its exact wild progenitor remains unidentified. Its adaptability to diverse climatic conditions, with along its culinary, medicinal and economic significance, has made onion one of the most essential crops

globally. However, they prefer loamy soil that is fertile, well drained and high in organic matter, with a preferable pH range between 6.0 and 8.0 (Olani and Fikre, 2010).

The quality of onion depends on shape, size, colour and pungency of bulbs. Onions are typically categorized into three types based on their skin colour *i.e.*, red, yellow and white. The red and yellow hues are due to 'anthocyanin' and 'quercetin' pigments respectively. The pungency of onions is primarily caused by the volatile oil allyl propyl disulfide, while their flavour is linked to compounds such as propyl disulfide, other disulfides and their sugar content. Variations in flavour, pungency and dry matter are crucial quality factors for onions used in processing. Onions are also rich in chemicals like phenolic flavonoids, which possess anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties. Notably, quercetin plays a significant role in enhancing disease resistance (Slimestad *et al*., 2007). In India, red onions with a strong pungency are flavoured, while the European and Japanese markets prefer the milder, less pungent yellow or white skinned varieties.

The nutritive value of onion varies by variety per 100 grams of the edible portion. Onions provide 40 kcal of energy, 9.34 g of carbohydrates, 4.024 g of sugar, 1.7 g of fiber, 0.1 g of fat and 1.1 g of protein with 89.1 g of water. They contain essential vitamins, including B1 (0.046 mg), B2 (0.027 mg), B3 (0.116 mg), B5 (0.123 mg), B6 (0.12 mg) and C (7.4 mg), along with minerals like magnesium (10 mg), manganese (0.12 mg), calcium (23 mg), phosphorus (29 mg), potassium (146 mg) and zinc (0.17 mg) (Maini *et al*., 1985). Onions are grown for food, religious purposes and as a key ingredient in spices and condiments. They are widely used in kitchens and industries, particularly for dehydration into onion flakes and powder, which are in high demand.

The onion crop is grown well in the area where rainfall is 500-550 mm. It does not thrive when the average rainfall exceeds 750-1000 mm during monsoon period. The ideal temperature for vegetative growth is 12.8-23.0 °C. For bulb formation, it requires long days and still higher temperature (20-25 °C). Onion is basically a long day crop but has been adapted to short day conditions under north indian plains (Choudhary, 2018).

China and India are the leading onion growing countries followed by USA, Egypt, Turky, Pakistan, Sudan, Bangladesh, Iran and Russia. India being the second largest producer of onion, accounts for 22.83 per cent of worlds onion production. In India onion is being grown in an area of 1541 thousand hectares with total production of 24.26 Million Tonnes and productivity of 15.75 MT/ha (Anon., 2023).

In Gujarat, onions cultivated in approximately 79.25 thousand hectares, yielding a total production of 2057 MT with an average productivity of 25.96 MT/ha (Anon., 2023).

Onion crop is affected in many infections disease. Among them, the major fungal diseases are; Anthracnose leaf spot (*Colletotrichum gloeosporioides* [Penz.] Penz. & Sace. in Penz.), Smudge (*Colletotrichum circinans* [Berk.] Voglino), Purple blotch (*Alternaria porri* [Ellis] Cif) and *Stemphylium* leaf blight and stalk rot (*Stemphylium vesicarium* [Wallr.] E. G. Simmons), bacterial diseases such as Bacterial flower stalk and leaf necrosis (*Pantoea agglomerans* [Beijerinck] Gavini *et al*.), viral diseases Yellow dwarf (Yellow dwarf virus) and nematode diseases stem and bulb nematode (*Ditylenchus dipsaci* [Kuhn] Filipjev) and root knot nematode (*Meloidogyne incognita* [Kofoid & White] Chitwood) (Lowell *et al*., 2012). Among these diseases, the Anthracnose leaf spot (*Colletotrichum gloeosporioides*)/Twister (*Colletotrichum* spp.) is one of the major constrains in onion cultivation (Hill, 2008).

The twister disease is caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and its perfect stage is *Glomerella cingulata*. The disease is also called as onion anthracnose or *Colletotrichum* blight or severe curls disease. The disease was first time reported near Zaria, North Nigeria, in 1969 (Ebenebe, 1980). In india, twister disease was first time reported from Satara district of Maharashtra in 1981. In Karnataka, this disease was first time reported in 1987 and was believed to be caused by *G. cingulata*, during *kharif* season.

The typical twister disease symptoms are initial small, whitish oval to irregular sunken spots on the leaves which extends further, followed by the appearance of concentric rings of acervuli in sunken necrotic spots. Die back is observed on severely affected leaves which leads to collapse of plants (Ebenebe, 1980). The losses estimated are 50-100 per cent in several fields which results in lower supply of onion in the market leading to higher prices. Rotting of bulbs may occur in severe cases, in field as well as during storage. Infection shows curling, twisting of leaves, chlorosis, abnormal elongation and twisting of neck portion with blight as well as dieback, scanty root system with galls and showing fungal growth (Patil *et al*., 2018).

To manage the disease, application of fungicides as spray is a common practice. However, the traditional fungicides need to be applied repeatedly. Now a days new fungicide molecules are available in the market which are having more efficacy against the fungal pathogens even at lower doses. In recent years, as the twister disease is emerging as an epidemic in most of the onion growing agro-climatic zones across the country. It is felt necessary to study about the pathogen for its successful management.

9. PRACTICAL UTILITY OF THE RESEARCH PROBLEM:

Onion is a crucial commercial crop in India, cultivated in three seasons: *Rabi*, *Kharif* and late *Kharif*. While *Rabi* season contributes 60 per cent of production, *Kharif* and late *Kharif*

account for 40 per cent (Sharma and Chauhan, 2024). Onion cultivation exists across various agro-climatic conditions, with systematic research started in 1960 (Singh, 1997). Foliar diseases are a major constraint to onion production, causing significant yield losses of 40-75 per cent without fungicide use (Pethybridgea *et al*., 2016).

Twister disease of onion became a major constraint in successful crop production in some areas of Saurashtra. The disease incidence increasing year after year in moderate to severe form causing poor yield of crop. Dutta *et al*. (2024) recorded 80-100 per cent yield loss due to this disease. Looking to the importance of disease, the present study aimed with assessing losses caused by them as well as impact of weather parameters on disease development and management through biorationals with follow objectives.

10. OBJECTIVES

1. To study the cultural and morphological characteristics of [(*Colletotrichum gloeosporioides)* (Penzig) Penzig and Saccarado].
2. To study the effect of weather parameters on twister disease of onion.
3. To assess the yield losses in onion due to twister disease.
4. To evaluate fungicides for the management of twister disease of onion under field condition.
5. To evaluate biorationals for the management of twister disease of onion under field condition.

11. REVIEW OF LITERATURE

In India, onion is a key spice and vegetable crop, but it is susceptible to various pathogens, including fungal, bacterial and viral diseases, leading to significant economic losses. In recent years, anthracnose leaf spot or twister disease of onion, caused by *Colletotrichum gloeosporioides*, has emerged as a major threat across the country, with severe outbreaks reported over the past few years. However, there is limited historical information on this disease, although data on *Colletotrichum* induced diseases in other crops are available. Therefore, this review consolidates the limited existing knowledge on onion twister disease alongside information on anthracnose in other crops caused by *Colletotrichum* spp. The literature available on twister disease of onion and other related crops pertaining to its economic importance, isolation, purification, identification, symptomatology, pathogenicity cultural characteristics, morphological characteristics, epidemiology, yield losses, evaluation of fungicides and biorational against the disease under field condition is reviewed in this chapter.

11.1 Economic importance

Dutta *et al.* (2022) reported that anthracnose-twister disease of onion, primarily a rainy season disease, was first identified near Zaria in northern Nigeria in 1969. The disease was recognized as highly destructive, with projected yield losses ranging from 50 to 100 per cent under favourable environmental conditions. This highlights the severe economic impact of the disease in onion-growing regions, particularly during high humidity and rainfall periods.

Saravanan *et al.* (2023) identified *Colletotrichum gloeosporioides* as the causal agent of twister blight, which poses a significant threat to onion cultivation in Tamil Nadu. The disease was first reported in 1969 from Zaria, northern Nigeria (Ebenebe, 1980) and later in India from Lonand, Satara district, Maharashtra, in 1981. Since then, it has spread to several onion-growing regions across the country. In Tamil Nadu, the disease has been associated with severe yield losses ranging from 50 to 100 per cent, highlighting its destructive potential under conducive environmental conditions.

Dutta *et al.* (2024) reported that the anthracnose-twister is very important during *Kharif* (rainy) season and onions are highly susceptible to the anthracnose-twister, which is currently the most devastating disease causing estimated yield losses of onion to the tune of 80 to 100 per cent, depending on its severity and the growth stage of the crop.

Mahalakshmi *et al*. (2024) reported occurrences of twister blight disease in major onion-growing regions of Tamil Nadu. The disease has led to substantial yield losses, ranging from 50 to 100 per cent, highlighting its destructive nature. The economic threat posed by the disease under favourable conditions.

11.1.1. Isolation, purification and identification of pathogen

Patil (2013) conducted a comprehensive study on onion twister disease, particularly its occurrence in Karnataka, India. Patil's work detailed the purification process using both morphological and molecular techniques, identifying the causative pathogens as *Colletotrichum gloeosporioides, Fusarium oxysporum, Meloidogyne* spp.*, C. acutatum, Glomerella acutata* and *Gibberella moniliformis*. His research established that amplification of the ITS rDNA region and sequencing could confirm the pathogens' identity, while PCR–RFLP with the HaeIII enzyme differentiated species. Provided one of the first integrative approaches for the comprehensive identification and management of twister disease in India

Kimaru *et al.* (2018) taken samples of anthracnose infected avocado fruits from a research location in Kenya and the fungus was recovered using a tissue isolation approach. For future usage, pure cultures were stored in PDA slants at 4 °C in the refrigerator. Fungal isolates were identified as *Colletotrichum gloeosporioides* and *Pestalotiopsis* spp. based on morphological characteristics.

Alpeshkumar (2022) isolated the causal organism from symptomatic onion plants displaying typical twister disease symptoms such as curling, twisting, and chlorosis of leaves, along with necrotic lesions on the neck and bulb. The pathogen was isolated on PDA medium, where it produced colonies that were cottony to dirty white or yellowish-pink, flat to fluffy with regular to irregular margins. The colony later turned grey to black due to acervuli formation.

11.1.2. Symptomatology and pathogenicity

Gautam (2014) described onion anthracnose symptoms caused by *C. gloeosporioides* as sunken, water-soaked spots that rapidly expanded. As lesions matured, they became soft, reddish-brown to tan or black and varied in shape from round to oval or irregular.

Bajad (2017) demonstrated the pathogenicity of *Colletotrichum gloeosporioides* on one month old onions cultivar Basavant-780 by spray inoculation method. Based on typical symptoms on the foliage, characteristics of the fungal culture, microscopic observations and pathogenicity tests, the fungus was identified as *C. gloeosporioides* causing anthracnose on onions.

Patil *et al*. (2018) conducted studies during *Kharif* and *Rabi*/Summer seasons of 2011–12 and 2012–13, which revealed typical symptoms of onion twister disease, including leaf twisting, neck blight, dieback (anthracnose), a scanty root system with galls and visible fungal growth. Artificial inoculations confirmed that *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Meloidogyne* spp., both individually and in combination, could reproduce twister disease symptoms. Pathogenicity tests supported the complex etiology of the disease, leading to the proposal of a model disease cycle for the onion twister disease complex.

Dutta *et al.* (2022) observed onion anthracnose symptoms beginning as small, whitish, water-soaked lesions on leaves. These lesions later expanded into dark, oval, necrotic areas with concentric spore masses containing salmon or orange coloured acervuli, which eventually turned brown to black with setae. In severe cases lesions also appeared on onion bulbs leading to bulb rot and ultimately plant death.

Saravanan *et al*. (2023) reported that infected onion plants exhibited a range of symptoms, including chlorosis, leaf twisting and curling, formation of acervuli in the neck region and the development of slender bulbs. The pathogen isolated from the infected foliage produced greyish white to grey mycelium, which was hyaline and septate in nature.

Dutta *et al*. (2024) demonstrated that both *Colletotrichum gloeosporioides* and *Fusarium acutatum* independently cause anthracnose-twister symptoms in onion, with *C. gloeosporioides* inducing twisting and *F. acutatum* causing neck elongation. GA_3 alone

increased plant height without symptoms, but in combination with both pathogens, it intensified disease severity, indicating a synergistic effect. Acervuli were observed on *C. gloeosporioides* infected tissues and Koch's postulates were confirmed.

11.1.3. Cultural characteristics of *Colletotrichum gloeosporioides*

Asalkar *et al.* (2019) studied the growth and sporulation of *C. gloeosporioides* on various solid media. The highest growth and sporulation were observed on Richard's Agar (90 mm), followed by PDA (88.83 mm) and Corn Meal Agar (88.40 mm), while the least growth was recorded on Host Extract Dextrose Agar (68.17 mm).

Marak *et al.* (2019) studied the growth characteristics of *C. truncatum*, which causes anthracnose in green gram, on various media. Maximum growth (90 mm) was observed on PDA, Potato Carrot Agar, Carrot Agar, and Richard's Agar at 10 days post-inoculation. The least growth was recorded on Sabouraud's Agar (69.56 mm) and V-8 Juice Agar (55.42 mm).

Udhayakumar *et al.* (2019) investigated the morphological and cultural characteristics of *C. gloeosporioides* isolates and observed that most produced greyish-white colonies on PDA. Pigmentation varied among isolates, with colours ranging from whitish and greyish-brown to greenish-grey, pinkish and pinkish-brown. Additionally, the isolates differed in their ability to produce conidia on PDA.

Lopes *et al.* (2021) evaluated the morphological characteristics and growth rate of nine fungal isolates by placing 4 mm discs from five-day-old colonies onto PDA medium. The experiment, conducted twice with three replicates in a completely randomized design, involved incubation at 25 °C under a continuous photoperiod. Colony diameter was measured daily for seven days in two perpendicular directions using a digital calliper and mycelial growth was expressed in mm/day. At the end of the incubation period, colony colour and texture were assessed to classify the isolates into distinct morphological groups.

Reecha *et al*. (2022) noted that *Colletotrichum gloeosporioides* colonies isolated from shallots turned dark brown over time. Microscopic examination revealed abundant, oval to elongated conidia measuring approximately 13 µm in length and 3.52-3.89 µm in width, typically non-septate, characteristic of the *C. gloeosporioides* group.

Syafitri *et al*. (2023) reported that symptomatic shallot leaf samples collected from Bantul, Yogyakarta an important shallot-producing region in Indonesia were cultured on PDA medium. Infected plants showed leaf drying, visible lesions on bulbs and eventual bulb rot leading to plant death. The fungal colony initially exhibited white to grey mycelium, which darkened with age.

Yu *et al*. (2024) showed that *Colletotrichum spaethianum* and *C. circinans* were cultured on 1.5% PDA for 3 days, followed by sub culturing on 1.5% malt extract agar at 25 °C for 7 and 14 days, respectively.

11.1.4. Morphological characteristics of *Colletotrichum gloeosporioides*

Gautam (2014) reported that the mycelium of *C. gloeosporioides* was hyaline, septate and branched. The conidia were hyaline, single-celled, ovoid to oblong, straight or slightly curved, with obtuse spices, measuring 10-15 µm in length and 5-7 µm in width.

Murtado *et al*. (2020) identified *Colletotrichum* sp. and *Fusarium* sp. as the pathogenic fungi associated with wilted onion leaves. The fungal structures, including spores and setae, were observed adhering to the surface of withered leaves under 100X magnification. The colony of *Colletotrichum* sp. appeared yellowish-orange to white with thin, septate, hyaline hyphae. The conidia were generally cylindrical in shape, measuring approximately 3-5 × 9-14 µm.

Dutta *et al.* (2022) reported that *C. gloeosporioides* produced hyaline, single-celled conidia that were ovoid to oblong, slightly curved or dumbbell-shaped with obtuse ends. These conidia developed on well-formed conidiophores.

Saravanan *et al.* (2023) reported that the pathogen isolated from infected onion leaves exhibited greyish white to grey mycelium with hyaline, septate hyphae. The conidia were cylindrical, hyaline and measured 15-20 µm in length and 4-7 µm in width. These morphological characteristics are typical of *Colletotrichum* spp., aiding in the identification and confirmation of the causal agent of onion twister blight disease.

Dutta *et al.* (2024) observed that *Colletotrichum* spp. colonies were white to grey with yellowish-orange pigmentation, bearing septate, hyaline mycelium and cylindrical to dumbbell-shaped conidia (3.6-5.4 × 10.5-15.3 µm). Acervuli with orange conidial masses confirmed the species. *Fusarium* spp. showed white colonies with aerial hyphae, septate mycelium, curved macroconidia (17.8 × 3.1 µm), ovoid aseptate microconidia (4.3 × 2.1 µm) and single, intercalary chlamydospores.

11.2 To study the effect of weather parameters on twister disease of onion.

Patil and Nargund (2016_a) conducted study to find out the relationship between weather factors and the severity of onion twister disease. Weather factors have a direct and significant effect on disease incidence. Further study showed that disease progressed was at a linear rate throughout crop growth. The correlation coefficient revealed that the mean cumulative rainfall contributed maximum (r = 0.93) to the development of the disease in relation to other parameters followed by maximum temperature (r = 0.84). The coefficient of determination (R^2)

value for pooled data equation was 0.97. This could be attributed to the high temperature associated with the relative humidity which increased the inoculum level. During study, the maximum disease severity in the Karnataka coastal region of Uttara Kannada was mainly attributed to the extreme climatic season with temperatures between 28 and 36 °C, soil type and cultivation mode.

Rao *et al.* (2021) recorded the relationship between various environmental factors and field bean anthracnose (*Colletotrichum lindemuthianum*). The results revealed that progress of disease increased slightly from first week of November to last week of January or first week of February in both years thereafter declined gradually due to raise in the temperature. During the major progress, mean maximum and minimum temperature were around 29.49 °C and 20.13 °C, respectively whereas morning relative humidity was 86.05 per cent with moderate rainfall. Correlation analysis showed positive significant correlation of per cent disease index with maximum temperature and sunshine hours; non-significant correlation with minimum temperature and evaporation; significant negative correlation with evening relative humidity and rainfall. Multiple correlation co-efficient indicated strong relationship between disease severity and weather variables, thereby, contributed more than 95.10 per cent variation during 2016-17 and 93.90 per cent variation during 2017-18. The prediction equations developed using multiple linear correlation were, $Y = -3.802 + 5.154X+0.01X_2$ with $R^2 = 0.984$ and $Y = -2.135+1.637X-0.031\ X_2$ with R^2-0.989 for 2016-17 and 2017-18, respectively which helps in planning and execution of forecasting.

Sohaliya (2021) reported that severity of anthracnose of black gram has significant correlation with weather parameters. Weather parameters *viz.*, maximum and minimum temperature, morning and evening relative humidity, rainfall, sunshine hours and wind velocity has important role in anthracnose development. It is favoured by cool and humid condition with temperature range of 22 to 29 °C. In 2019, maximum temperature and wind velocity were found non-significant while minimum temperature was found significant whereas morning and evening relative humidity were found positively significant in the year 2020. Maximum temperature and minimum temperature were found non-significant. While, morning relative humidity, evening relative humidity and wind speed were found positively significant with anthracnose disease of black gram.

The effect of weather parameters on anthracnose disease development in green gram was studied for the year 2019, 2020 and 2021 by Nandeesha (2021). He reported that prevailing moderate temperature of 25-30°C, high relative humidity (>90%) in combination with intermittent rainfall found essential for disease establishment as observed in all three seasons.

Correlation study, rendered negative correlation for maximum temperature (-0.542), highly significant negative correlation for minimum temperature (-0.772) and wind speed (-0.841). However, positive correlation was observed for evening relative humidity (0.304), sunshine hour (0.734) and rain fall (0.360). While, significantly positive correlation was observed for morning relative humidity (0.608). Multiple linear regression model obtained was, $Y = 790.37 - 6.70X_1 - 13.49X_2 + 0.86X_3 - 3.43X_4 - 7.47X_5 + 4.24X_6 + 0.18X_7$ with high coefficient of determination $R^2 = 0.95$.

11.3 To assess the yield losses in onion due to twister disease

Dutta *et al.* (2022) reported that anthracnose-twister disease in onion, caused by *Colletotrichum gloeosporioides*, can lead to yield losses of up to 100 per cent in unmanaged fields. The disease is intensified by interactions with gibberellin producing fungi like *Gibberella moniliformis*, resulting in symptoms such as leaf twisting, acervuli formation, and bulb rot. The study highlights the need for integrated disease management involving cultural, biological and chemical control strategies to reduce losses effectively.

Dutta *et al.* (2024) evaluated the cumulative impact of *Colletotrichum gloeosporioides* and *Fusarium acutatum* in causing the anthracnose twister disease complex of onion, with a particular focus on disease progression and its implications for yield. Their controlled pot experiments revealed that while *C. gloeosporioides* primarily induced leaf twisting and *F. acutatum* caused neck elongation, their co-infection significantly intensified symptom severity. Importantly, the interaction led to earlier disease onset and greater physiological stress on the plant, resulting in marked reductions in plant biomass and potential yield. The study underscored that this synergism further amplified by exogenous gibberellic acid (GA3) could lead to substantial yield losses under field conditions, particularly when left unmanaged. These findings reinforce the necessity of integrated strategies that simultaneously address multiple pathogens and hormonal imbalances to mitigate economic losses in onion production.

Jameela and Balabaskar (2024) conducted comprehensive field surveillance in Tamil Nadu to assess the prevalence and severity of onion twister blight caused by *Colletotrichum gloeosporioides*. Their surveys, conducted during the *Kharif* seasons of 2020 and 2021, recorded PDI values for twister disease ranging from 12 to 35 per cent, compared to purple blotch PDI of 8 to 23 per cent. The study highlighted that twister blight, often occurring as a complex with *Fusarium* spp. and nematodes, was more pervasive and damaging than purple blotch. In untreated field plots, PDI reached up to 49 %, whereas integrated treatments such as *Trichoderma* soil drenches combined with foliar applications of tebuconazole or azoxystrobin successfully reduced PDI to as low as 14%. These findings underscore the critical impact of

environmental conditions and disease complexes on disease severity and emphasize the value of integrated management strategies in reducing disease incidence and protecting yield potential.

11.4 To evaluate fungicides for the management of twister disease of onion under field condition.

Gyempeh *et al.* (2015) showed that *Trichoderma asperellum* effectively suppressed the mycelial growth and sporulation of *Colletotrichum gloeosporioides*, the causal agent of onion anthracnose. Disease incidence was recorded at 43.6 % in Kwahu South and 62.5 % in Fanteakwa districts, with corresponding severity levels of 0.8 and 2.7 on a 1-5 scale. The causal agent was confirmed as *Colletotrichum gloeosporioides.* The bio-agent, *T. asperellium* was able to significantly suppress the mycelial growth and sporulation.

Patil and Nargund (2017) conducted a field trial to evaluate the efficacy of fungicides and insecticides against onion twister disease. The fungicides tested included mancozeb (0.25%), propiconazole (0.1%), copper oxychloride (0.25%), tricyclazole (0.1%) and hexaconazole (0.1%), both alone and in combination with insecticides such as methomyl (0.8 g/L), carbosulfan (2 ml/L) and profenophos (1 ml/L). Three sequential sprays were applied at 30, 45 and 60 days after transplanting (DAT) at 15-day intervals. The results indicated that sequential sprays of mancozeb (0.25%), tricyclazole (0.1%) and hexaconazole (0.1%) resulted in the lowest PDI (29.58), followed by mancozeb (0.25%), propiconazole (0.1%) and copper oxychloride (0.1%) (43.54). The highest PDI (61.63 %) was observed in the untreated control.

Perez and Alberto (2020) reported that protective spray applications of captan and paclobutrazol resulted in the lowest incidence of twister disease in onions. Meanwhile, onions treated with carbendazim and paclobutrazol exhibited the lowest disease severity under both protective and curative spray applications. In contrast, onion plants treated with difenoconazole, propiconazole and auxin showed the highest disease incidence and severity. The highest yield was recorded in plants treated with carbendazim + paclobutrazol and captan, while the lowest yield was observed in onions treated with auxin and difenoconazole, propiconazole. The study concluded that protective spray applications led to lower disease incidence and severity, shorter neck length and higher yield compared to curative sprays, affirming that chemical management remains the most effective approach for controlling onion twister disease.

Manthesha *et al.* (2022) evaluated fungicides and bio agents against twister disease of onion and reported that among the different treatments, foliar application of carbendazim 12 + mancozeb 63 WP at 2.5 g/L, *Trichoderma* + *Pseudomonas* mixture at 10 g/L and propiconazole

25 EC at 1 ml/L resulted in the lowest PDI (16.59, 17.26 and 18.54, respectively). The highest PDI (72.11) was observed in the control plot. The study emphasized that spraying any of these fungicides or bio agent mixtures three times at 15-day intervals after the first symptom appearance is essential for effective disease control.

Dutta *et al*. (2022) evaluated the effectiveness of 13 different *Trichoderma* spp. against onion anthracnose-twister disease. The results indicated that all tested isolates significantly improved crop growth parameters, including a 4-8% increase in leaf diameter, 6-27% increase in pseudo-stem girth and 9-19% increase in the number of leaves. Disease incidence reduced by 6-48%, while bulb yield increased by 5-7%. Among the treatments, *Trichoderma* T-4 R and *T. harzianum* showed the best results, followed by NRCGT-8 and *T. asperellum*. The combined foliar application of *Trichoderma* and *Pseudomonas* (1% talc formulation) were also effective in suppressing disease severity. In disease management, *Trichoderma* treatments enhanced crop biomass, yield and bulb shelf life. Although chitin has been reported to manage anthracnose in other crops and improve growth and nutrient content in onion bulbs, it has not yet been tested against onion anthracnose.

11.5 To evaluate the biorationals for the management of twister disease of onion under field condition

Gowthamchand *et al.* (2020) carried out an experiment during *kharif* 2018-19 at Organic Farming Research Centre, Zonal Agricultural and Horticultural Research Station, Shivamogga on French bean (*Phaseolus vulgaris* L.). They reported that the maximum protein content in seed was registered with application of *beejamrut* (seed treatment) + *jeevamrut* (soil application @ 500 lit/ha) + 100% RDN through vermicompost + foliar spray of *panchagavya* @ 3%.

Kumar *et al*. (2022) recorded that in response to the adverse impacts of intensive agricultural practices introduced during the Green Revolution, farmers have increasingly shown interest in organic and natural farming systems. Among the prominent organic inputs, *Jeevamrit* (also spelled *Jeevamrutha*) has emerged as a highly effective and low-cost microbial formulation traditionally prepared from on-farm inputs such as cow dung, cow urine, jaggery and pulse flour. It functions as a potent soil microbial enhancer, being rich in beneficial microorganisms including bacteria, fungi and actinomycetes. The application of *Jeevamrit* not only enhances soil microbial activity and improves nutrient availability but is also believed to stimulate crop growth and help in repelling certain insect pests, thereby playing a dual role in promoting plant health and protecting crops in natural farming systems.

11.5.1 Biochemical parameters

Alberto (2014) studied pathogenesis in three onion cultivars and found that uninoculated onion plants had higher protein content than inoculated plants. Biochemical analyses revealed that among the three cultivars inoculated with *Colletotrichum gloeosporioides*, the lowest protein levels were observed in Yellow Granex (10.68%), Red Creole (9.58%) and Shallot (13.74%). Among the infected cultivars, protein content was highest in Shallot and lowest in Yellow Granex. Total soluble sugars and reducing sugars were also higher in the resistant cultivars. Interestingly, sugar levels decreased after infection, suggesting a correlation between increased sugar content and plant resistance. Furthermore, uninoculated leaves contained relatively low levels of phenol, whereas phenol accumulation increased after infection. In resistant genotypes, total phenol accumulation was frequently higher in diseased leaves than in susceptible ones, though an overall increase in phenol levels was observed across all genotypes as the disease progressed.

Patil and Nargund (2016) observed biochemical changes in onion due to twister disease in the Dharwad region. Their study revealed that reducing sugars, non-reducing sugars and total sugars were highest in plants at the fifth stage of disease intensity (severely infected plants) with values of 8.74, 6.68 and 15.54 mg/g of leaf tissue, respectively. Conversely, total protein content was highest at the first stage of disease intensity (mildly infected plants) with a recorded value of 6.34 mg/g of leaf tissue.

12. MATERIALS AND METHODS

The present investigation on twister disease of onion will be carried out during 2025-26 and 2026-27 in the laboratory and experimental fields of Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh. The details of various materials used and the methodology followed are described as under.

12.1 To study the isolation, purification, identification, symptomatology and pathogenicity of [(*Colletotrichum gloeosporioides*) (Penzig) Penzig and Saccardo] causing twister disease of onion.

12.1.1 Collection of diseased plant parts

The diseased plant showing twister caused by *Colletotrichum gloeosporioides* will be collected from experimental plots of Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh. The freshly collected diseased leaves of twister/ anthracnose in onion showing typical symptoms like as chlorosis, curling and twisting of the onion leaves, abnormal elongation of the necks and acervuli formation of *C. gloeosporioides* on the necks and leaf blades of diseased plants will be collected in brown paper bags from onion fields during 2025-26. It brought the laboratory and sample will be thoroughly washed

under running tap water and then immediately examined under a compound microscope for presence of the pathogen. Later on, the fungus will be isolated and brought into pure culture of potato dextrose agar.

12.1.2 Isolation and purification

Severely infected leaves of onion showing typical disease symptoms of twister/leaf spot on leaf, neck and root will be used for the isolation of fungus from the infected plants. The isolation of the pathogens causing twister of onion from infected leaves will be followed by standard tissue isolation technique (Tuite, 1969). Diseased plant parts will be cut in small pieces (2-3 mm) along with some healthy tissues using sterilized scalpel, these pieces will be surface sterilized with NaClO (1 %) for 30 seconds and rinsed thrice in distilled sterilized water. Then, it will be dried using sterilized blotting paper and aseptically transferred to sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) media. The plates will be incubated at 27 ± 2°C. After 4 days, the hyphal growth will be cut and transferred to other PDA plates. The plates will be observed periodically for fungal growth and sporulation. Then it will be purified by single spore isolation technique.

12.1.3 Identification of pathogen

Identification of pathogens causing twister disease of onion grown on media will be examined visually as well as microscopically for cultural and morphological characters

12.1.4 Maintenance of culture

The isolated pure culture of fungal pathogen grown on PDA media will be transferred to 10 ml culture slant by hyphal tip technique and regular sub culturing of fungus will be done at an interval of 25 - 30 days and incubated in the dark at 27 ± 2 °C for seven to eight days until the surface of PDA cover with a dense growth of fungal culture The culture tubes will be labeled and stored at 4 °C. This culture will be sub-cultured at regular intervals and used for further examination of research work.

12.1.5 Cultural characteristics

Isolated pathogen previously purified through single spore, maintained by hyphal tip technique on PDA slants and stored in refrigerator (4 °C) will be separately grown on PDA in Petri plates for cultural study. The cultural characters will be rerecorded right from the initiation of growth up to 10 days incubated at 27 ± 2 °C including colony colour, topography of colony, colony margin, colony diameter and colony pigmentation.

12.1.6 Morphological characteristics

A small amount of culture of test pathogen obtained from ten days old culture will be placed on the slide and teased thoroughly with lactophenol blue to obtain uniform spread. A

cover slip will be placed over it. The morphological characters *viz*., length and breadth of 20 conidia and setae will be measured under high power magnification (400X) digital microscope loaded with camera from 10 days old culture of pathogen and average sizes will be calculated. Morphological studies of test pathogen will be conducted on PDA media. Spore characteristics such as colour, size and shape will be recorded primarily with the help of simple compound microscope. Identification will be carried out by comparing the microscopic and morphological features of the pathogenic fungi with the available standard literature.

12.1.7 Symptomatology

The visual and microscopic examination will be carried out for showing twister disease symptoms like chlorosis, curling and twisting of the onion leaves, abnormal elongation of the necks and acervuli of *C. gloeosporioides* on the necks and leaf blades of diseased plants to confirm the presence of pathogens.

12.1.8 Pathogenicity

The pathogenicity test of *C. gloeosporioides* will be proved by artificial inoculation of pathogens by spray inoculation technique (Koch's postulates). Pathogenicity tests will be carried out to establish that the fungi isolated from infected plant tissues is capable of producing typical symptoms of twister under artificial inoculation conditions on onion.

The pathogenicity will be proved under pot conditions using healthy seed of onion variety. Surface sterilized seeds will be sowed in 30 cm diameter earthen pot seed bed containing mixture of sterilized black soil: sand: farm yard manure (3:2:2 by volume) under controlled condition. The soil will be sterilized in autoclave at 121 °C and 15 lbs psi for 15 minutes. Simultaneously, the pots will be sterilized with 4 per cent formalin solution and autoclaved soil will be filled in these pots. Thirty days old seedlings of onion cultivar previously grown in a seedbed, will be transplanted into earthen pots containing 5 kg mixture of sterilized soil @ 5 plants per pot and replicated four times. At 20 days after transplanting, spray inoculated plant with 5 ml spore suspension ($1x10^6$ cfu/ml min.) using an atomizer in the morning until run-off will be covered with polythene bag immediately to maintain high humidity. Spore suspensions of fungal culture will be prepared by harvesting the spores from 10-12 days old pure culture growth on PDA media. This suspension will be used for inoculation. The cultures multiplied on PDA will be scraped using a sterilized surgical scalpel and transferred into 10 ml sterilized distilled water under aseptic conditions. The test tubes will be shaken for 15 minutes, to get a uniform, homogenized spore/mycelia suspension ($1x10^6$ cfu/ml min.) and to break the mycelial clumps if any.

The control pot without inoculation will be sprayed with sterilized distilled water. The inoculated and uninoculated pots will be covered with polyethylene bags for 48 hr to provide high humidity. The observations on the disease development on leaves will be recorded periodically from the initiation of the disease. Re-isolation will be performed from the artificially inoculated plants showing typical symptoms of twister disease by tissue isolation method. The culture obtained by re-isolation will be transferred on PDA slants for comparison with original culture and further investigations.

12.2 To study the effect of weather parameters on twister disease of onion.

Onion will be transplanted to main field during late *Kharif*-2025 adopting common package of practices. Data for first date of appearance of twister and gradual progress on the crop will be monitored and observations on per cent disease intensity will be recorded at regular interval of four days till harvest of the crop.

Weather data on maximum and minimum temperatures, morning and afternoon relative humidity, wind speed, bright sunshine hours, rainy days and rainfall (mm) will be collected from Agrometeorological Cell, Department of Agronomy, JAU, Junagadh and utilized for establishing relationship of weather parameters with disease development. Different ranges of weather variables of seven days preceding the assessment date will be used as independent variables to identify the boundary and favourable conditions that influenced the dependent variables of twister disease severity on the crops following initial infection, through regression analysis.

The onion variety “Agri Found Dark Red” will be sown in nursery raised seed bed and thereafter transplanted in the main field at the age of 35-45 days in the plot size of 10.0 m x 10.0 m area for disease development. The per cent disease intensity will be calculated by adopting 0 to 5 disease rating grade given by Patil *et al*. (2013) for twister disease of onion as per given in Table 1.

The per cent disease intensity (PDI) will be calculated by using the following formula (Wheeler, 1969).

$$\text{Per cent Disease Intensity (PDI)} = \frac{\text{Sum of the individual disease ratings}}{\text{No. of leaves observed} \times \text{Maximum rating scale}} \times 100$$

The weather parameters will be correlated at four days disease severity index by calculating the Karl Person’s correlation coefficient (r). Correlation coefficient values will be tested individually for their significance at 5% probability level using following formula:

$$t = \frac{r\sqrt{(n-2)}}{\sqrt{1-r2}}$$

Where, t = Test of significance

r = Correlation coefficient and

n = Number of observations

Table 1. Disease rating grade for assessment of disease severity against twister disease of onion (Patil *et al*., 2013).

Disease rating (grade)	Twisting	Description
0	No disease	No disease symptom
1	Up to 10%	Curling and chlorosis leaves
2	11-20%	Abnormal elongation of leaves and neck
3	21-40%	Leaf sheath showing clusters of acervuli in concentric rings along with shallow, sunken necrotic spots and root galling
4	41-60%	Elongation neck, slender bulbs and leaves show dieback symptoms
5	>61%	Severe dieback, rotten bulbs, root system under developed with discoloured roots

Statistical analysis:

Further, multiple regressions will be carried out with weather parameters in relation to PDI. The relationship of disease with weather parameters will be established by following multiple linear regression equation.

$Y = a+b_1X_1+b_2X_2+b_3X_3+b_4X_4+b_5X_5+b_6X_6+b_7X_7+b_8X_8$

Where,

Y = Disease intensity

a = Intercept (constant)

b_1 to b_8 = Partial regression coefficient associated with each x_i

i = 1, 2, 3, 4, 5, 6, 7, 8 are the weather variables

X_1 = Maximum temperature (^{0} C)

X_2 = Minimum temperature (^{0} C)

X_3 = Morning relative humidity (%)

X_4 = Afternoon relative humidity (%)

X_5 = Wind speed (km / hr.)

X_6 = Bright sunshine hours (hr.)

X_7 = Rainy days (mm)

X_8 = Rainfall (mm)

12.3 To assess the yield losses in onion due to twister disease

A field trial will be conducted using Paired t-test design during *Kharif* season of 2025-26 and 2026-27 at Research Farm, Department of Plant Pathology, JAU, Junagadh using onion variety Agri Found Dark Red. For this purpose, crop will be sown in two plots of 24 m x 25 m size with 15 x 10 cm spacing. One plot will be sprayed with each of the benzimidazole, triazole and strobilurin group of fungicides at recommended dose first at the time of initiation of the disease, thereafter required sprays at twelve days interval to maintain disease free condition. The remaining unsprayed plot will be kept as control. The observations will be taken from each treatment at four days interval.

The disease incidence will be recorded by following the 0-5 scale (Patil *et al*., 2013) as mentioned in the Table 1 from 10 randomly selected plants from each sample and five leaves from each plant will be selected after seven days of last spray. The bulb yield will be recorded after harvest of crop from both sprayed and unsprayed plot. The bulb yield from each sub of the size 5.4 m × 5.0 m will be drawn from all the sub plot at maturity of crop. The yield determined for the protected plot will be taken as a base for computing the losses. Paired t-test will be used for statistical analysis. The losses in yield will be calculated as per formula given by Gupta and Singh (1981).

$$\text{Per cent loss in yield} = \frac{\text{Yield of treated plot - Yield of untreated plot}}{\text{Yield of treated plot}} \times 100$$

Percent Disease Intensity (PDI)

The disease intensity will be recorded by scoring on individual ten plants using 0 -5 scale. Further the PDI will be calculated using the formula given earlier in 12.2.

12.4 To evaluate fungicides for the management of twister disease of onion under field condition

a) Location : Research Farm, Department of Plant Pathology, COA, JAU, Junagadh

b) Crop : Onion

c) Year : Late *Kharif*: 2025-26 and 2026-27

d) Experimental design : Randomized Block Design

e) Treatments : Seven

f) Replications : Three

g) Plot size : 5.0 m x 1.80 m

h) Seed rate : 10 (kg/ha)

i) Spacing : 15 x 10 cm

j) Fertilizer dose : 100:50:50 NPK (kg/ha)

k) Observation to be recorded : 1. Disease intensity (%)
2. Bulb yield (t/ha)

Table 2. Treatment details and application methods for field evaluation of fungicides for the management of twister disease of onion (Anon., 2024)

Module	**Treatment details**	**Application method and time**
Module 1	Seed treatment with Tebuconazole 25.9 EC @ 1 mL/lit and soil application of FYM enriched *Trichoderma harzianum* @ 2.5 kg /250 kg FYM	Seed treatment at nursery
	Tebuconazole 25.9 EC @ 1 mL/lit while transplanting also apply FYM enriched *T. harzianum*	Root dipping at transplanting
	Tebuconazole 25.9 EC @ 1 mL/lit and soil application of FYM enriched *T. harzianum* @ 2.5 kg /250 kg FYM	Foliar spray at 30-35 DAT
	Azoxystrobin 18.2 + Difenoconazole 11.4 SC @ 1.25 mL/lit	Foliar spray at 45 DAT
	Pyroclostrobin 5 + Matiram 55 WG @ 0.9 mL/lit	Foliar spray at 60 DAT
Module 2	Seed treatment with Tebuconazole 25.9 EC @ 1 mL/lit and soil application of FYM enriched *Trichoderma harzianum* @ 2.5 kg /250 kg FYM	Seed treatment at nursery
	Carbosulfan 25 EC @ 2 mL/ lit + Tebuconazole 25.9 EC @ 1 mL/lit while transplanting also apply FYM enriched *T. harzianum* @ 2.5 kg/250 kg	Root dipping at transplanting
	Tebuconazole 25.9 EC @ 1 mL/lit and Fipronil 5 SC @ 1 mL/lit, soil application of FYM enriched *T. harzianum* @ 2.5 kg /250 kg FYM	Foliar spray at 30-35 DAT
	Azoxystrobin 18.2 + Difenoconazole 11.4 SC @ 1.25 mL/lit and Carbosulfan 25 EC @ 2 mL/lit	Foliar spray at 45-50 DAT
	Pyraclostrobin 5 + Metiram 55 WG @ 0.9 mL/lit and Profenofos 50 EC @ 1 mL/lit	Foliar spray at 60 DAT
Module 3	Seed treatment with Carboxin 37.5 + Thiram 37.5 DS @ 2 g/kg seeds	Seed treatment at nursery
	Hexaconazole 5 EC @ 1 mL/lit + Spinosad 45 SC at 0.25 mL/lit	Foliar spray at 30 DAT
	Hexaconazole 5 EC @ 1 mL/lit + Spinosad 45 SC at 0.25 mL/lit	Foliar spray at 60 DAT

Module 4	Seed treatment with *T. harzianum* 1 WP and soil application of FYM enriched *Trichoderma harzianum* 1 WP @ 2.5 kg /250 kg FYM	Seed treatment at Nursery
	FYM enriched *T. harzianum* 1 WP @ 2.5 kg /250 kg FYM and mulching with straw	Soil application during TP
	Pseudomonas fluorescens 1 WP (20 g/lit) + *Beauveria bassiana* 1.15 WP @ 3 - 4 g/lit.	Foliar spray at 30 DAT
	Azadiractin 1500 ppm (0.15 EC) @ 3-4 mL/lit and *P. fluorescens* 1 WP (20 g/lit) + *B. bassiana* 1.15 WP @ 3-4 g/lit	Foliar spray at 45 DAT
	Azadiractin 1500 ppm (0.15 EC) @ 3-4 ml/lit. and *P. fluorescens* (20 g/lit)+*B. bassiana* 1.15 WP @ 3-4 g/lit	Foliar spray at 60 DAT
Module 5	Soil application of *T. viride* 1 WP @ 5.0 kg/ha + spray of Fluopyram 17.7 + Tebuconazole 17.7 SC (400 SC) @ 0.2 (2 mL/lit)	at 15 DAT
	Azoxystrobin 18.2 + Difenoconazole 11.4 SC @ 0.1 (1.25 mL/lit)	Foliar spray at 30 DAT
	Azoxystrobin 18.2 + Cyperoconazole 7.3 w/w SC (280 SC) @ 0.2 (2 mL/lit)	Foliar spray at 45 DAT
	Tebuconazole 50 + Trifloxystrobin 25 WG (75 WG) @ 0.1 (1 g/lit)	Foliar spray at 60 DAT
	Pyraclostrobin 5 + Metiram 55 WG @ 3 g/lit	Foliar spray at 75 DAT
Module 6	Existing Practice of location. Soil application of chlorantraniliprole 0.4 G (10 kg/ha) and copper oxychloride 50 WP (1.25 kg/ha)	At the time of transplanting
	Sprays of tebuconazole 50 + trifloxystrobin 25 WG 0.0075% (10 g/10 lit water)	At initiation of disease
	Sprays of azoxystrobin 18.2 + difenoconazole SC 0.03% (10 g/10 lit water)	At 15 days after first spray
Module 7	Absolute Control	-

Methodology

Above module based treatments will be given as per specified in Table 2. All the recommended agronomical practices will be followed during experimentation. Onion seeds will be sown in raised nursery beds of the size 5.0 m x 2.0 m in field condition. The fungicidal suspension will be used @ 500 litre/ha/spray.

Disease intensity (%)

The disease intensity will be recorded seven days after last spray. Ten plants will be selected randomly and labelled from each plot for recording the disease intensity. The selected plants will be graded using the 0-5 disease rating grade as per Table 1. Further, the PDI will be calculated using the formula given earlier in 12.2.

12.5 To evaluate biorationals for the management of twister disease in onion under field condition.

a) Location : Research Farm, Department of Plant Pathology, COA, JAU, Junagadh

b) Crop : Onion

c) Year : Late *Kharif*: 2025-26 and 2026-27

d) Experimental design : Randomized Block Design

e) Treatments : Seven

f) Replications : Four

g) Plot size : 5.0 m x 1.80 m

h) Seed rate : 10 (kg/ha)

i) Spacing : 15 x 10 cm

j) Fertilizer dose : Not applicable

k) Observation to be recorded : 1. Disease intensity (%)
2. Bulb yield (t/ha)
3. Biochemical parameters
   a) Protein
   b) Phenol
   c) Sugar

Table 3. Treatment details (Acharya, 2020).

Sr. No.	Treatment	Concen-tration (%)	Quantity of formulation in 10 lit of water	a.i. (g/ha)	Quantity of formulation lit/ha
1.	*Jivamrut*	5.0	500	-	25.0
2.	*Beejamrut*	5.0	500	-	25.0
3.	Sour butter milk	10.0	1000	-	50.0
4.	Neem leaf extract	5.0	50.0	-	25.0
5.	Cow urine	3.0	300	-	15.0
6.	Sonthashtra	5.0	500	-	25.0
7.	Control	-	-	-	-

Methodology:

Biorationals will be prepared as per standard protocol (Acharya, 2020). Whereas, Neem leaf extract will be prepared following the method given by Agbenin and Marley (2006). All the recommended agronomical practices will be adopted for raising the crop. The foliar sprays will be done as per the treatments. The suspension of biorationals will be applied as foliar spray

@ 500 lit/ha. First spray will be given at the initiation of the diseases and subsequent spray will be given at an interval of 10 days.

Disease intensity (%)

The disease intensity will be recorded seven days after last spray. Ten plants will be selected randomly and labelled from each plot for recording the disease intensity. The selected plants will be graded using the 0-5 disease rating grade as per Table 1. Further, the PDI will be calculated using the formula given earlier in 12.2.

12.5.1 Biochemical Parameters

The glass wares will be scrubbed and washed thoroughly with detergent, washed with tap water followed by distilled water. Finally, the glass wares will be dried in an oven before use. All poly wares will be thoroughly cleaned as stated above and air dried before use. All the stated chemicals and reagents used will be analytical grade purity and obtained from standard made.

For studying biochemical changes in *C. gloeosporioides* infected plants, plant part will be collected at 80 days after transplanting. Uniform leaves with respect to age, size and shape will be selected and used. Analysis for the estimation of protein, phenol and total soluble sugar will be carried out using standard methods as given below.

12.5.1.1 The total sugar

The total sugar content will be determined by colorimetric method as suggested by Dubois *et al*. (1956).

12.5.1.1.1 Reducing sugar

Reducing sugar content will be measured following Nelson's modification of "Somogyi's method" (Somogyi, 1952) using arseno-molybdate colour forming reagent and two copper reagent A and B.

12.5.1.1.2 Non-reducing sugar

The amount of non-reducing sugar will be obtained by subtracting reducing sugar from the number of total sugars and multiplying the resultant with a constant factor 0.95.

12.5.1.2 True protein estimation

True protein content in plant extract will be checked by using the Folin-Lowry's method (Lowry *et al.*, 1951).

12.5.1.3 Total phenol estimation

The total phenol content will be estimated by the method described by Bray and Thorpe (1954).

13. Statistical analysis

Statistical analysis will be carried out as per standard method (Panse and Sukhatme, 1985).

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